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**A qualitative investigation of major urinary proteins in relation to the onset  
of aggressive behavior and dispersive motivation in male wild house mice  
(*Mus musculus domesticus*)**

Rusu, A S ; Krackow, S ; Jedelsky, P L ; Stopka, P ; König, B

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DOI: <https://doi.org/10.1007/s10164-007-0042-3>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-2311>

Journal Article

Published Version

Originally published at:

Rusu, A S; Krackow, S; Jedelsky, P L; Stopka, P; König, B (2008). A qualitative investigation of major urinary proteins in relation to the onset of aggressive behavior and dispersive motivation in male wild house mice (*Mus musculus domesticus*). *Journal of Ethology*, 26(1):127-135.

DOI: <https://doi.org/10.1007/s10164-007-0042-3>

# A qualitative investigation of major urinary proteins in relation to the onset of aggressive behavior and dispersive motivation in male wild house mice (*Mus musculus domesticus*)

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Received: 13 June 2006 / Accepted: 24 March 2007 / Published online: 10 May 2007  
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**Abstract** The physiological basis for population differentiation of dispersal timing during individual development in male wild house mice is still unknown. As major urinary proteins (MUPs) are known to convey information about competitive ability in male mice, we examined individual MUP profiles defined by isoelectric-focusing (IEF) patterns in relation to developmental timing of dispersive motivation. As an experimental paradigm marking the development of the dispersal propensity, we used agonistic onset between litter mate brothers when kept in pairs under laboratory conditions. Agonistic onset is known to reflect the initiation of dispersive motivation. Hence, we compared individual MUP IEF patterns between fraternal pairs that did or did not develop agonistic relationships before the age of 2 months. Urine was collected on the day of weaning and at the beginning of adulthood. We investigated whether there was a significant co-occurrence of particular MUP IEF patterns with the agonistic onset in male mice. We assumed that, based on this co-occurrence, particular MUP IEF patterns and/or a particular dynamic of MUP IEF expression from weaning to adulthood may be considered a physiological predictor of a specific behavioral strategy in

male mice (i.e. submissive-philopatric or agonistic-dispersive strategy). We found that agonistic males expressed more MUP IEF bands than amicable ones at weaning, but these differences disappeared later on. The presence of two particular IEF bands at weaning was significantly associated with early agonistic onset. Our study suggests that MUPs could have a predictive value for the onset of aggressive behavior and dispersal tendency in male wild house mice.

**Keywords** Dispersal propensity · Chemical signals · Aggressive behavior · Wild house mice

## Introduction

Competitive aggression and dispersal propensity represent behavioral traits of paramount importance to individual fitness in animals (Anderson 1990; Johnson and Gaines 1990). At 2–3 months of age, commensal male mice (*Mus musculus domesticus*) frequently engage in overt aggressive interactions with other group members (i.e., litter mates and full or half-siblings) and disperse unless they are able to overtake the natal territory themselves (Lidicker 1976; Van Zegeren 1980; Stenseth and Lidicker 1992; Gerlach 1996). The temporal coincidence during ontogeny of the agonistic onset and dispersal propensity suggests that these two behavioral traits represent parts of the same behavioral syndrome in wild house mouse males (Rusu and Krackow 2005).

We used a laboratory paradigm marking the emergence of dispersal propensity in wild house mouse males reared in pairs of brothers from the day of weaning to their adulthood. This paradigm originates from the observation that, under semi-natural conditions, the social relationships of young males with their same-sex litter mates are

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amicable until males decide to disperse from their natal deme (i.e., family-based group) starting at about 2 months of age. Young males then abruptly engage in overt agonistic interactions and subsequently leave their natal deme unless they are able to defeat the territorial male (Gerlach 1996). This temporal coincidence of agonistic onset and dispersal suggests that both behavioral tendencies might represent components of the same behavioral syndrome. That is, males would initially follow a *submissive-philopatric* strategy and later switch to an *agonistic-dispersive* strategy (Rusu and Krackow 2005). When reared in fraternal pairs under laboratory conditions, males typically engage in agonistic relationships around 2 months of age (Krackow 2005), which is reminiscent of the timing in semi-natural enclosures. In congruence, males are significantly more prone to dispersing from a social group after agonistic onset than before agonistic onset when they prefer to adopt a submissive status within their deme (Rusu and Krackow 2005). Hence, agonistic onset qualifies as a paradigm for the ontogenetic timing of the dispersive propensity in male house mice.

To identify a possible physiological predictor of which behavioral strategy (i.e., submissive-philopatric or agonistic-dispersive strategy) a male mouse will follow during its development, we chose to investigate the qualitative patterns of major urinary proteins (MUPs) excreted in different moments of individual ontogeny, such as time of weaning and beginning of adulthood. Proteins in urine predominantly consist of MUPs that bind and release aggression-mediating volatile pheromones (Novotny et al. 1985; Cavaggioni et al. 1990; Bacchinni et al. 1992; Bocskei et al. 1992; Hurst et al. 1998) and themselves act as chemosignals in the process of individual recognition in male mice (Hurst et al. 2001; Nevison et al. 2003).

In addition to their role in individual recognition, MUPs are reliable signals of competitive abilities of successfully territorial males (Mucignat-Caretta et al. 2004; Hurst and Beynon 2004). Moreover, a recent study shows that territorial male owners always countermark urine from mice of different genetic background, regardless of their major histocompatibility complex (MHC) similarity and familiarity (Hurst et al. 2005). This suggests that the MUP urinary profile could be the best candidate for signaling the genetic background, and thus the fixed characteristics, of an individual, including its propensity to follow a specific behavioral strategy.

The MUP urinary profile of each individual is stable over its life span, and it is not altered by changes in social status, diet or illness (Hurst et al. 2001). Substantial daily urinary MUP excretion of 20–40 mg clearly indicates the paramount importance of MUPs to chemical communication in house mice (Hurst et al. 2001; Robertson et al. 1997; Beynon and Hurst 2003). Indeed, MUPs can affect traits

such as mate recognition, pregnancy block, puberty acceleration, estrus induction and counter-marking behavior and are now known to occur in the urine of both sexes of wild house mice (Beynon and Hurst 2004). Patterns of MUP expression are particularly relevant to agonistic ontogenetic development given that they are known to be testosterone-dependent (Knopf and Held 1980; Clissold et al. 1984), gene-expression inducers (Brennan and Peele 2003), and agonistic-response mediators (Hurst et al. 2001; Mucignat-Caretta et al. 2004).

We aimed to investigate whether MUP urinary profiles, which were studied by isoelectric focusing (IEF), could predict the behavioral trajectory that a male mouse would follow during its development. We compared individual MUP IEF patterns of males from fraternal pairs that had initiated agonistic interactions before the age of 2 months (i.e., agonistic fraternal pairs) with IEF patterns of males that had not developed aggression before that age (i.e., amicable fraternal pairs). We collected urine samples at weaning (day 21) and at 2 months of age (day 61), assuming that differences in MUP IEF patterns could indicate the physiological switch from submissive-philopatric strategy to aggressive-dispersive strategy.

We did not aim here to identify the mechanism by which various MUP patterns could influence aggressive and dispersive behavior. Rather, we attempted to explore whether there was any significant co-occurrence of particular MUP IEF patterns with behavioral phenomena, such as agonistic onset and dispersal motivation in male mice. Based on this co-occurrence, particular MUP IEF profiles and/or a particular dynamic of MUP production during development (i.e., from weaning to adulthood) may be considered as a physiological predictor of a specific behavioral strategy in male mice.

## Materials and methods

### Animals

Experimental animals were offspring from opportunistically outbred descendants of 30 reproductive pairs of wild-caught mice (*Mus musculus domesticus*,  $2n = 24$  chromosomes) originating from four Swiss lowland feral populations. Mice were bred monogamously under standard laboratory conditions (Perspex Macrolon cages of  $26.5 \times 42 \times 15$  cm; 12:12 h light:dark cycle with lights on at 0600 hours;  $22 \pm 1^\circ\text{C}$ ; 50–60% relative humidity). Pups were weaned at 21 days of age and placed into fresh cages with same-sex litter mates (groups of 2 or 3 animals per cage), except for experimental males, which were transferred into fresh cages in fraternal pairs. The fraternal pairs were left undisturbed until 61 days of age.

### Agonistic data collection

For data acquisition, 28 pairs of litter-mate brothers screened for agonism at 61 days of age in the course of another experiment (Rusu and Krackow 2005) were sampled for urine, as outlined below. Details of experimental determination of agonistic status are given in Rusu and Krackow (2005). In short, males were judged to have established agonistic relationships when exhibiting scarring of tail and/or back of body. Males not exhibiting clear signs of an established agonistic relationship were subjected to an aggression test. In these cases, brothers were separated and placed into individual cages ( $22 \times 36 \times 15$  cm) for at least half an hour. Subsequently, both cages were connected to a clean cage using plexiglass tubes (4-cm diameter). Social behavior was recorded for 15 min following the first contact of the two individuals. Fraternal pairs were categorized as “agonistic” when agonistic interactions occurred (i.e., attack, bite, chase, flee, approach/retreat and fight; Mackintosh 1981). Non-agonistic mice were termed “amicable.”

### MUP analysis

Urinary samples were taken at weaning (day 21) and before the agonistic test (day 61) from 56 males (28 males of 14 agonistic pairs, and 28 males of 14 amicable fraternal pairs). With the exception of two pairs of brothers (one agonistic pair and one amicable pair) that came from the same litter, the fraternal pairs were unrelated to each other. Each individual was held by the back of the neck and by the tail base over a clean commercially available waterproof PVC plate (transparent, rigid and 3-mm thick) for 1–2 min. The PVC plates were cut to perfectly fit the bottom of the mouse-handling box in our laboratory in Zurich (i.e. dark Perspex Macrolon box of  $35 \times 48 \times 45$  cm). Each mouse had its own PVC plate, and all the plates were cleaned at the end of each urine collection session. After the handling, the mouse was returned to its cage, and the PVC plate was removed from the handling box. Urine was aspirated directly from the plate using a Pasteur pipette and transferred to a micro-centrifuge tube. Tubes were stored at  $-20^{\circ}\text{C}$  until further analysis was performed. Prior to electrophoresis, the total protein concentration of each sample was determined with the Bradford assay (Bio-Rad) using the protocol described in Stoscheck (1990). For each male mouse, the total urinary protein concentrations (mg/ml) at days 21 and 61 are given in Table 1.

### Isoelectric focusing

Isoelectric focusing (IEF) was performed on commercially available precast Immobiline Dry Plates pH 4.2–4.9

(Amersham Biosciences) on a Multiphor II Flatbed Electrophoresis system (Amersham Biosciences). According to the protocol described by Westermeier (2001), the gels were rehydrated for 60 min in 20 mM acetic acid (Sigma). Urine samples were diluted 1:10 with deionised water, and 10- $\mu\text{l}$  aliquots were loaded onto the gel using Sample Application Strips (Amersham Biosciences), which were positioned 2 cm from the acidic edge of the gel. The gels were run for 20 kVh (maximum voltage set to 3,000 V, maximum power 5 W and cooling  $10^{\circ}\text{C}$ ) and then immediately stained for 30 min with staining solution (0.5% Coomassie Brilliant Blue R-250, 0.5% Crocein Scarlet in 30% ethanol and 10% acetic acid). The gels were destained overnight in 30% ethanol and 10% acetic acid.

A pI (position in millimeters from the acidic edge of the IEF gel) value for each IEF band was estimated using PDQuest Software (Bio-Rad). In estimating the pI values, it was assumed that the edges of the gel were 4.2 and 4.9, respectively, and the gradient was linear. Also, a male C57BL urine sample was used as reference. Each IEF band was assigned a number that represented the distance (mm) from the acidic edge of the gel. Figure 1 illustrates an example of a gel with four individual samples showing the maximum number of IEF bands and their estimated pI values. An intergel comparison was accomplished using an arbitrary sample that was used as a standard in every gel. Resulting IEF patterns were visually scored by staff unaware of individual behavioral phenotypes. The presence of individual bands was transcribed into a binary matrix (0, absence; 1, presence).

### Mass spectrometric analysis

Following IEF separation, a representative of each band type was analyzed by peptide mass fingerprinting on MALDI MS. Proteins subjected to mass spectrometric analysis were digested “in gel” according to the protocol used by Volf et al. (2002). Resulting peptides were extracted from the gel by increasing acetonitrile concentration to 30%; after 15 min of sonication, they were subjected to MALDI MS analysis.

A mixture of one part of saturated solution of  $\alpha$ -cyano-4-hydroxycinnamic acid in methanol and one part of 45% acetonitrile and 5% acetic acid was used as a matrix solution. The sample aliquots (total volume 0.5  $\mu\text{l}$ ) were loaded onto the sample target and left to dry. Dried spots were covered with 0.5- $\mu\text{l}$  drops of matrix solution and left to crystallize. Positive ion MALDI mass spectra were recorded on a Bruker BIFLEX II reflectron time-of-flight mass spectrometer (Bruker–Franzen, Bremen, Germany) equipped with a SCOUT 26 sample inlet, a gridless delayed

**Table 1** Urinary protein concentration and number of IEF bands for each male

Amicable					Agonistic				
ID	Day 21		Day 61		ID and status	Day 21		Day 61	
	No. IEF bands	Protein (mg/ml)	No. IEF bands	Protein (mg/ml)		No. IEF bands	Protein (mg/ml)	No. IEF bands	Protein (mg/ml)
1.1	8	9.1	8	29.8	15.1d	7	10.1	10	26.9
1.2	8	9.8	9	28.9	15.2s	9	9.9	9	29.3
2.1	8	9.7	9	30.1	16.1s	9	9.7	10	27.6
2.2	7	8.8	9	29.7	16.2d	10	9.4	10	28.8
3.1	9	9.7	10	28.8	17.1d	11	9.8	11	28.7
3.2	7	9.7	8	26.5	17.2s	13	10.2	13	29.6
4.1	8	9.6	11	29.7	18.1s	9	8.7	10	28.8
4.2	9	9.8	11	29.7	18.2d	12	9.1	10	29.7
5.1	8	9.5	10	28.9	19.1d	10	9.7	11	29.7
5.2	9	10.1	10	29.4	19.2s	13	9.6	12	29.3
6.1	10	10.2	9	28.9	20.1s	14	9.8	15	29.2
6.2	9	9.8	8	29.1	20.1d	12	9.9	10	28.9
7.1	11	9.7	12	29.3	21.1d	11	9.8	12	27.8
7.2	10	9.9	10	29.4	21.2s	12	9.5	11	30.2
8.1	8	9.1	11	28.4	22.1s	14	10.1	12	29.4
8.2	10	9.5	11	28.7	22.2d	12	10.2	11	29.7
9.1	9	9.4	10	29.2	23.1d	13	9.5	11	27.8
9.2	10	9.6	11	29.3	23.2s	15	9.7	13	29.9
10.1	10	9.8	12	27.8	24.1s	9	10.1	10	28.9
10.2	9	9.9	10	30.2	24.2d	12	9.3	11	29.1
11.1	10	9.7	11	31.1	25.1d	9	9.7	10	29.3
11.2	11	8.9	11	28.8	25.2s	16	10.2	13	27.8
12.1	9	9.3	9	28.1	26.1s	11	9.7	10	28.9
12.2	9	9.9	10	27.3	26.2d	13	9.6	12	29.3
13.1	10	9.1	12	29.1	27.1s	12	9.8	11	28.8
13.2	11	9.8	11	28.9	27.2d	11	9.4	10	29.8
14.1	9	9.8	12	29.3	28.1s	12	9.9	11	30.1
14.2	10	9.4	11	27.5	28.2d	9	9.8	11	28.9

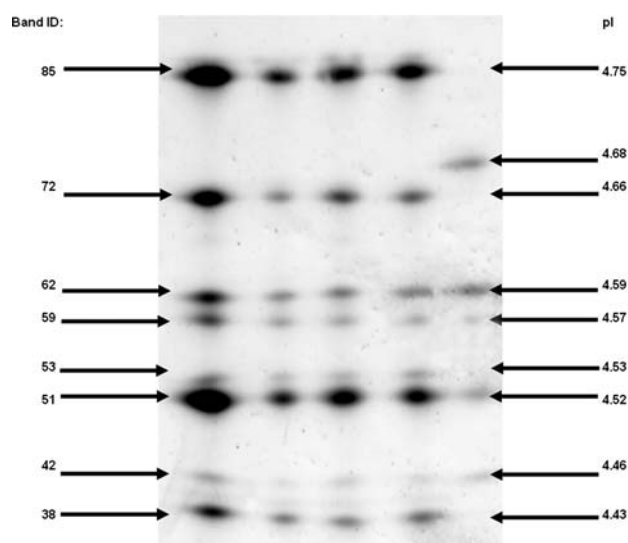
Number of IEF bands observed in each agonistic and amicable male at 21 and 61 days of age and value of total urinary protein concentration (mg/ml) estimated for each sample. Each pair of brothers was given an ID number (from 1 to 28) and within the pair each brother was numbered as 1 or 2 (i.e., 1.1 and 1.2). For the agonistic pairs of brothers, we also added the social status of each individual, which is either *d*, dominant, or *s*, submissive

extraction ion source, and a 337-nm nitrogen laser (Laser Science, Cambridge, MA, USA). The ion acceleration voltage was 19 kV, and the reflectron voltage was set to 20 kV. Spectra were calibrated externally using the mono-isotopic  $[M + H]^+$  ion of somatostatin I (Aldrich). The identification of the resulting peptide mass fingerprints was achieved using Peptide Mass Fingerprint of Mascot (<http://www.matrixscience.com>). According to this identification procedure, all the analyzed IEF bands were MUPs. However, the MUP polymorphism did not allow a detailed classification.

#### Statistical analysis and data interpretation

We compared the total number of IEF bands occurring per fraternal pair as well as the number of bands unshared by brothers, between amicable and agonistic pairs, with a Wilcoxon Mann–Whitney rank–sum (*S*) test (equivalent to Mann–Whitney *U* test). We chose the fraternal pair for independent data points, as there was no indisputable evidence as to which male initiated agonism during ontogeny, if it occurred. We also tested for individual change in IEF band number and change in fraternal disparity from day 21





**Fig. 1** Example of an IEF gel with male urine samples. The gel contains four individual samples of male urine showing the maximum number of MUP IEF bands. For each IEF band, the estimated pI value and the position in millimeters from the acidic edge are indicated on the figure. The *far right lane* represents the IEF urine pattern of a C57B16 male that was used as a standard

to 61 using the Wilcoxon signed-rank matched-pairs test (yielding the signed-rank sum,  $S_p$ , test statistics). Strong deviation from normality prevented parametric testing.

Multiple logistic regression was performed to identify effects of the occurrence of IEF bands on agonistic phenotype of fraternal pairs. This implies binomial error distribution for the response (agonistic/amicable) and use of a logit link function, as well as correction of effect parameters for any other effect in the model. Each IEF band was represented by an independent variable containing the number of males exhibiting the respective IEF band in a fraternal pair (0–2). Twelve bands exhibited sufficient variation (Table 2), while the remaining bands were either present in or absent from fewer than three fraternal pairs, which did prevent meaningful analysis (i.e., the maximum likelihood algorithms did not converge). Stepwise logistic regression included effects with  $P < 0.1$  until no further effects added to the model. From the final model, significances were derived based on model deviances (log-likelihood ratio chi-squares).

We are aware of the fact that a specific IEF band may contain more than one MUP, and that slight variation in procedure and/or preparation can change IEF positions (pI) of identical MUPs between probes, resulting in under- and overestimation, respectively, of the actual number of MUPs in a sample (Robertson et al. 1996). However, neither of those potential procedural inadequacies would affect inferences to be drawn from our analyses. First, nearly all IEF bands occurred more often in agonistic than amicable males (cf. Table 2). Hence, a larger number of

IEF bands necessarily means a larger number of different MUPs in our sample. Second, we analyzed samples in identical fashion, and agonistic and amicable males' urine samples, as well as day 21 and 61 samples, were not analyzed in different batches but in random sequence. We therefore consider procedural effects as random with respect to our designed effects.

## Results

We observed 25 bands with different pI values and different IEF locations in the urinary samples collected from 14 pairs of agonistic brothers and 14 pairs of amicable ones. A total of 13 bands were either absent or present in two or fewer pairs and were excluded from logistic analysis. No significant correlation was found between the protein concentration of each sample and the number of IEF bands produced at days 21 and 61.

Most agonistic fraternal pairs exhibited 11 IEF bands at weaning (day 21), significantly more than amicable pairs, which exhibited a mode of 8 ( $S_p = 139$ ,  $z = 2.96$ ,  $P < 0.004$ ; Table 3). There was no significant difference at 2 months of age when both agonistic and amicable fraternal pairs exhibited a modal excretion of 10 IEF bands ( $S_p = 178$ ,  $z = 1.15$ ,  $P > 0.24$ ; Table 3). Accordingly, males in amicable pairs exhibited and increased the number

**Table 2** IEF MUP bands expressed by the agonistic and amicable pairs of brothers

IEF band	mm	pI	Day 21		Day 61	
			Amicable	Agonistic	Amicable	Agonistic
62 <sup>a</sup>	4.59		10 (5)	14 (9)	10 (9)	13 (9)
59	4.57		10 (8)	12 (9)	12 (10)	11 (10)
53 <sup>a</sup>	4.53		8 (7)	13 (11)	10 (10)	12 (11)
72	4.66		8 (4)	12 (6)	13 (11)	14 (9)
42	4.46		8 (6)	11 (4)	14 (13)	14 (10)
74	4.7		7 (4)	10 (9)	6 (5)	10 (9)
76	4.71		3 (3)	6 (4)	4 (3)	5 (3)
40	4.44		2 (2)	6 (2)	3 (3)	1 (1)
54	4.55		5 (4)	3 (0)	4 (4)	3 (1)
63	4.61		2 (0)	6 (2)	3 (1)	5 (1)
57	4.58		5 (2)	2 (0)	4 (3)	1 (0)
52	4.51		1 (0)	2 (1)	1 (1)	3 (2)

Number of amicable pairs (out of 14) and agonistic fraternal pairs (out of 14) exhibiting bands of different IEF positions and at different distances (mm), on the isoelectric-focusing (IEF) gels, at 21 and 61 days of age. *Numbers in parentheses* refer to the number of pairs where both males' urine exhibited the respective band. Bands of very high and low prevalence are not shown

pI Position in millimeters from the acidic edge of the IEF gel

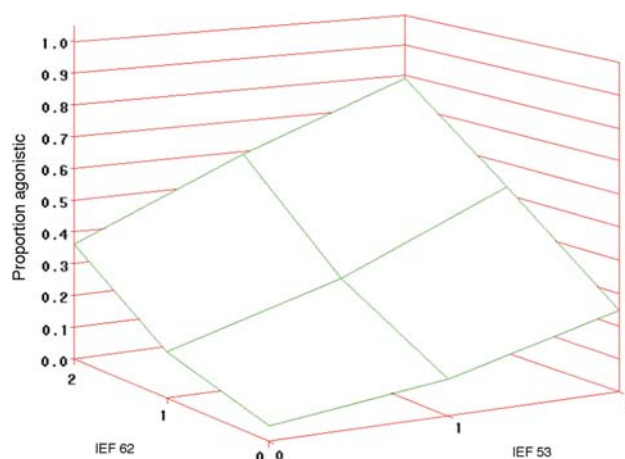
<sup>a</sup> Significantly differentiating bands

of MUP IEF bands from weaning to 2 months of age, while agonistic males had a constant number of IEF bands during development (Table 3). Brothers generally exhibited unshared IEF bands (Table 3), but the differences were not significantly sharper in one of the experimental groups at day 21 ( $S_p = 174$ ,  $z = 1.33$ ,  $P > 0.18$ ) or day 61 ( $S_p = 171$ ,  $z = 1.55$ ,  $P > 0.12$ ), nor did the number of unshared bands significantly change with age (amicable:  $S_p = 10.5$ ,  $P > 0.17$ ; agonistic:  $S_p = -5.5$ ,  $P > 0.42$ ).

Stepwise logistic regression demonstrated IEF band 62 ( $pI = 4.59$ ) and band 53 ( $pI = 4.53$ ) with significant effects on day 21 (model:  $\chi^2_2 = 8.26$ ,  $P < 0.02$ ; band 62:  $\chi^2_1 = 4.15$ ,  $P < 0.05$ ; band 53:  $\chi^2_1 = 3.54$ ,  $P < 0.06$ ; Fig. 2). It is noteworthy that in three amicable pairs, IEF band 62 was absent and in another five pairs IEF band 53 did not show up at all, whereas in one additional pair neither band was found at weaning (day 21). Among agonistic brothers, all pairs exhibited both bands, except one case where band 53 was missing. On day 61, no regressor was retained in the model, i.e., MUP IEF composition at 61 days of age did not differentiate agonistic phenotypes.

## Discussion

Given the importance of MUP release for social communication in house mice (Novotny et al. 1985; Hurst et al.



**Fig. 2** Predicted effect of two particular MUP bands at agonistic onset. Predicted proportion of fraternal pairs exhibiting agonistic phenotype as a function of the number of males excreting IEF MUP bands 62 and 53 at weaning (0 no brother of a pair excreted the band; 1 one brother excreted the band; 2 both brothers of a pair excreted the band)

1998, 2001; Mucignat-Caretta et al. 2004), our findings indicate that MUP IEF patterns can predict behavioral strategies related to aggression and dispersal in maturing male mice (*Mus musculus domesticus*). Males from both agonistic and amicable fraternal pairs exhibited different MUP IEF profiles. Amicable pairs exhibited fewer IEF bands than agonistic ones at weaning (day 21), but they subsequently gained IEF bands so that the two categories of males did not differ at 2 months of age.

The presence of both IEF bands 53 ( $pI 4.53$ ) and 62 ( $pI 4.59$ ) at weaning significantly increased the probability of agonistic onset before 2 months of age. Indeed, at day 21, at least one of the two bands was absent from 9 out of 14 amicable fraternal pairs, but only one was absent from 1 out of 14 agonistic pairs. This could indicate that the MUPs represented by these two IEF bands at least participated in communicating competitive status, although they were obviously not immediately effective in eliciting agonistic interactions (i.e., some of the amicable pairs excreted them at weaning, and MUP patterns were similar between agonistic and amicable fraternal pairs at 2 months of age). This could be explained in two ways. First, the changes in MUP patterns from day 21 to 61 could have exerted, with some delay, effects on agonistic behavior of males. Second, the congruent delay in MUP pattern development and agonistic onset timing in amicable males could be linked via correlated underlying mechanisms, although with no causal link between the two effects. The former interpretation implies that MUPs signal competitive abilities and initiate a process leading to the establishment of agonistic relationships. The second interpretation requires that MUP excretion is ontogenetically controlled

**Table 3** Comparisons of IEF MUP profiles between and within the agonistic and amicable categories of males

	<i>n</i>	Mean	SE	Q1	Med	Q3	$S_p$	<i>P</i>
Amicable pairs								
No. IEF, day 21 <sup>a</sup>	14	9.14	0.38	8	9	10		
No. IEF, day 61	14	10.21	0.32	9	10	11		
Bands gained	28	1.71	0.35	0	1.5	2.5	101.5	<0.001
Unshared, day 21	14	2.21	0.58	0	2	3	27.5	<0.002
Unshared, day 61	14	0.93	0.46	0	0	1	7.5	<0.1
Agonistic pairs								
No. IEF, day 21 <sup>a</sup>	14	11.43	0.59	11	11.5	12		
No. IEF, day 61	14	11.07	0.46	10	11	12		
Bands gained	28	0.04	0.58	-1	0	1	5.0	<0.9
Unshared, day 21	14	3.50	0.79	1	3	5	39.0	<0.001
Unshared, day 61	14	2.71	1.12	0	1	3	22.5	<0.004

Number of IEF bands observed in agonistic and amicable fraternal pairs at 21 and 61 days of age, number of bands gained per male from 21 to 61 days of age, and number of bands unshared between brothers at the two ages. Sample size (*n*), mean, standard error of the mean, first, second (median) and third quartile are shown. Where appropriate, Wilcoxon signed-rank matched-pairs test statistics ( $S_p$ ) for means equaling 0 are given

<sup>a</sup> Significantly different between agonistic and amicable fraternal pairs

and in a way coherent with male competitive status. Both possibilities are explored below.

### MUP signaling of competitive abilities

Urinary MUPs not only bind and release aggression-mediating volatile pheromones, but also act on their own as signals of competitive ability in male mice (Hurst and Beynon 2004). MUPs stimulate competitive countermarking in male–male interactions, even when the volatile status-signaling compounds are displaced from the proteinaceous part of mouse urine (Beynon and Hurst 2004; Humphries et al. 1999). Moreover, when direct contact with urine and thus the investigation of MUPs was prevented, males still investigated the volatiles of the marks, but failed to countermark (Nevison et al. 2003). In addition, at least some MUP variants appear to exhibit specificity for binding (Marie et al. 2001). Changes in MUP patterns might therefore stimulate changes in interactions between males, either in their own right or due to specific ligand presentation. Hence, it seems plausible that MUPs could act as chemical signals that modify intermale agonism during ontogeny.

Further studies are needed to reveal whether the two specific MUP IEF bands (62 and 53) can signal a specific behavioral strategy of the maturing male mice (i.e., agonistic-dispersive strategy vs. philopatric-submissive) early in ontogeny. If so, it would be necessary to reveal to whom the messages about specific behavioral strategies of the maturing males were addressed (i.e. males, females or both). Finally, another assumption would be that the signals were circulated between different age categories of individuals, such as young and old males within a deme. To answer these questions, further investigations are needed to test the behavioral reactions and/or preferences of different sex and age receptors toward urinary stimuli that do or do not contain the two IEF bands. If female mice showed preference for a specific IEF-type stimulus (with or without the two specific bands), it would make sense to further investigate the fitness benefits related to their preference. This would allow for inferences regarding the development of the two behavioral strategies in natural populations of wild house mice in relation to specific socioecological contexts.

Some amicable pairs exhibited as many IEF bands as agonistic pairs, and in particular, at day 21, IEF bands 62 and 53 were both present in 5 of the 14 amicable fraternal pairs, and no differences between amicable and agonistic pairs occurred at 2 months of age. This rules out any immediately effective link between MUP excretion changes and agonistic onset. However, specific MUPs might not elicit pre-existing response, but, alternatively, they might induce developmental processes themselves.

Gene-expression effects induced by external stimuli on neuroendocrine metabolism are ubiquitous and well known for many pathways, including differential testosterone degradation in the rat brain (Rosenbrock et al. 1999). Such a genetic induction mechanism could lead to delayed responses. We emphasize that more detailed studies are necessary to fully understand the role of MUPs in agonistic onset determination in developing male mice. Only knowledge of the exact identity and concentration of MUPs and their effects in recipients can ultimately resolve the issue. Our current results clearly indicate that this would be a promising effort.

### Developmental control of competitive status and MUP production—a matter of further research

An important issue to be investigated in the future is whether MUP excretion is ontogenetically controlled in a coherent manner with male competitive status. Testosterone (T) represents one of the main hormonal factors controlling MUP production (Knopf and Held 1980; Clissold et al. 1984), and T blood levels are elevated during early ontogeny in male mice that become aggressive later in life (Compaan et al. 1994). Accordingly, reduced numbers of MUPs excreted in amicable males might follow from lower T levels in those males compared to agonistic males. However, it is not known whether increased aggression as measured in Compaan et al. (1994) relates to earlier agonistic onset, as defined in our study. Also, we could not determine whether or not missing IEF bands indicated if the respective MUPs might not have been expressed at all. In the former case, testosterone levels themselves, with an overall effect on the number of members of the MUP family, could be responsible for the observed effect. In the case of differential expression, additional control mechanisms have to be postulated in relation to specific control of expression. That would be in agreement with findings of Payne et al. (2001), who demonstrated on individual samples that at a later age some MUPs appeared that were not present in males at 21–27 days. Hence, MUP profiles could undergo stepwise changes due to independent regulation of particular MUP genes.

### Conclusions

Our findings indicate that major urinary proteins (determined by isoelectric focusing) are a physiological predictor of behavioral strategies related to important life-history traits such as dispersal behavior and agonistic onset in maturing male mice (*Mus musculus domesticus*). This is a preliminary study that offers a good starting point for further investigations of the potential physiological mechanism of



MUP production in relation to the ontogenetic timing of dispersive behavior in wild house mice. Such a physiological course could allow house mouse populations to locally adapt to varying dispersal regimes. Future research would have to evaluate whether overall differences in testosterone metabolism cause deviations in MUP patterns during development or if more specific control mechanisms are involved in MUP production. Recent data reported by Novotny et al. (2007) are encouraging in this regard, showing that several testosterone-dependent urinary volatiles that bind with MUPs are influenced by genes of the major histocompatibility complex (MHC genes). Additionally, investigations are needed to clarify whether MUP production and agonistic behavior onset are linked by shared determining pathways or MUP production is causal to the ontogenetic timing of agonistic behavior.

**Acknowledgements** We are indebted to three anonymous reviewers for valuable comments on former drafts of this manuscript. Our thanks also go to Jari Garbely for help with any laboratory matter arising and to Alan G. McElligott and Marius I. Benta for reviewing the English. Support from German Research Foundation (Heisenberg programme KR1290/6) to SK, Swiss National Fond (research grant 3100–59609) to BK and SK, Grant Agency of the Czech Republic (grant no. 206/04/0493) to PS and PJ, and from Competence Center for Gender Studies in Zurich and from Romanian Ministry of Education and Research (grant CEEX no. 3210/14.10.05) to AR is kindly acknowledged.

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